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Add the following:

▲Scaffold Human Amniotic Membrane Allograft

DEFINITION

Scaffold Human Amniotic Membrane Allograft is derived from normal human placental amniotic tissue obtained from full-term pregnancies. The amniotic tissue is isolated and decellularized to remove cellular contaminants, resulting in an extracellular matrix with basement membrane. Scaffold Human Amniotic Membrane Allograft is composed predominantly of collagen and elastin. Tissues are obtained from sources that have passed applicable donor tissue eligibility requirements for relevant diseases, are processed, dehydrated, and terminally sterilized with e-beam irradiation. Scaffold Human Amniotic Membrane Allograft is supplied in a dry sheet form and is stored at ambient temperature.

IMPURITIES

• **Analysis of Residual Sodium Deoxycholate**

Solution A: Purified water liquid chromatography–mass spectrometry (LC/MS) grade

Solution B: Acetonitrile LC/MS grade

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	95	5
8	25	75
9	2	98
13	95	5

Diluent 1: Acetonitrile and purified water, both LC/MS grade (50:50), and 0.1% formic acid

Diluent 2: Isopropyl alcohol and purified water (90:10)

Diluent 3: 50% ethanol in phosphate buffered saline (PBS)

Lock mass stock solution: Add 2.5 mL of purified LC/MS grade water to a 10-mg vial of leucine enkephalin acetate to obtain a 4-µg/µL stock solution.

Lock mass solution: 2 ng/µL prepared as follows. Transfer a 10-µL aliquot of the *Lock mass stock solution* to a 20-mL volumetric flask, and add *Diluent 1* to volume.

Calibration solution: 5 mM sodium formate in *Diluent 2*

Sodium deoxycholate stock solution 1: Weigh a 25 ± 0.5-mg portion of sodium deoxycholate, add to a 50-mL volumetric flask, and dissolve in *Diluent 3* to obtain a 500-µg/mL solution.

Sodium deoxycholate stock solution 2: Dilute *Sodium deoxycholate stock solution 1* volumetrically at a ratio of 1:50 in *Diluent 3* to obtain a 10-µg/mL solution.

Sodium deoxycholate standard solution: Dilute *Sodium deoxycholate stock solution 2* volumetrically at a ratio of 1:5 in *Diluent 3* to obtain a 2-µg/mL solution.

Lithocholic acid stock solution 1: Weigh a 25 ± 0.5-mg portion of lithocholic acid, add to a 50-mL volumetric flask, and dissolve in *Diluent 3* using a sonicator for approximately 30 min to obtain an approximate 500-µg/mL stock solution.

Lithocholic acid stock solution 2: Dilute *Lithocholic acid stock solution 1* volumetrically at a ratio of 1:50 in *Diluent 3* to obtain an approximate 10-µg/mL solution.

Lithocholic acid standard solution: Dilute *Lithocholic acid stock solution 2* volumetrically at a ratio of 1:5 in *Diluent 3* to obtain a 2-µg/mL solution.

Spiked internal standard solution 1: Dilute *Sodium deoxycholate standard solution* and *Lithocholic acid standard solution* in *Diluent 3* at a ratio of 1:1:18.

Spiked internal standard solution 2: Dilute *Sodium deoxycholate stock solution 2* and *Lithocholic acid stock solution 2* in *Diluent 3* at a ratio of 1:1:3.

Sample: 2 mg of Scaffold Human Amniotic Membrane Allograft. Subdivide the test and control sample(s) to facilitate transfer into 20-mL vials.

Spiked sample: Spike the sample with 60 µL of *Lithocholic acid standard solution*. Dry under a stream of nitrogen for 2 h.

Spiked control sample: Spike the sample with 60 µL of *Spiked internal standard solution 2*. Dry under a stream of nitrogen for 2 h.

Chromatographic system

Mode: UPLC

Detector: UV 190–400 nm

Column: 50-mm × 2.1-mm; 1.7-µm packing L7

Column temperature: 35°

Flow rate: 0.3 mL/min

Injection volume: 5 µL

Run time: 15 min

Mass spectrometry instrumental conditions

Mass analyzer: Quadrupole time of flight (QToF)

Mode of MS ionization: Electrospray ionization, negative ion mode

MS mode: Sensitivity

Spectral range: 50–1500 *m/z*

Detector: Time of flight (ToF)

Lock mass: 554.26 Da

Capillary: 2.6 kV

Sampling cone: 30 V

Extraction cone: 4 V

Temperatures

Source: 140°

Desolvation: 450°

System suitability

Sample: *Spiked internal standard solution 1*

Suitability requirements

Percent relative standard deviation (% RSD) detection limit: NMT 15%

Quality control check: 70%–130% of theoretical concentration

Relative response factor: 0.5–1.3

Deoxycholate–molecular mass (M–H): 391.2848 ± 0.02 Da

Internal standard–molecular mass (M–H): 375.2899 ± 0.02 Da

Analysis

Samples: *Spiked sample* and *Spiked control sample*

Sample extraction: Add *Diluent 3* to each sample vial containing *Spiked sample* or *Spiked control sample* using a ratio of 1.2 mL of *Diluent 3* per 2 mg of the sample. For each vial, extract the sample at 37 ± 2° for 120 ± 12 min. After the extraction is complete, remove the sample from each extraction vessel using a clean inert article, such as a disposable glass Pasteur pipette. Store the extract at 2°–8°.

Sample analysis: Analyze the extracts using instrument conditions described. Analyze 6 replicates of *Spiked internal standard solution 1* and *Diluent 3* as solvent blank as appropriate. Perform quality control (QC) checks at least every 10 samples using *Spiked internal standard solution 1*.

Analysis with mass spectrometer: Change the QToF mass spectrometer settings as follows.

Capillary: 2.6 kV

Sampling cone: 30.0 V

Extraction cone: 4 V

Temperatures

Source: 140°

Desolvation: 450°

Desolvation gas flow: 750 L/min

Use the *Calibration solution* to calibrate the mass spectrometer for a mass range of 50–1500 Da in sensitivity mode using tune page settings and without collision energy. Set up lock mass using *Lock mass solution* with a capillary voltage of 2.6 kV in negative mode of ionization at 554.26 Da (M–H).

Calculate the ratio of the deoxycholate peak area to the lithocholic acid peak area from the *Spiked sample* and the *Spiked control sample*.

Acceptance criteria: The ratio of the deoxycholate peak area to the lithocholic acid peak area from the *Spiked sample* must be less than that from the *Spiked control sample*.

- **Residual Cell Analysis**

Harris hematoxylin stock solution: Dissolve hematoxylin[1] in alcohol and 20 mL of water. Add ammonium aluminum sulfate in water with heat until completely dissolved. Remove the ammonium aluminum sulfate solution from heat and mix with the hematoxylin solution. Bring the mixed solution to a rapid boil. Immediately remove from heat, add sodium iodate, and mix. Reheat the solution to boiling and boil for approximately 2–3 min. Remove from heat and plunge the solution container into an ice bath. Allow the solution to cool and pass through a grade 541 or comparable filter paper. Store the solution in a light-protected bottle at room temperature. This solution is stable for approximately 2 months.

Harris hematoxylin working solution: Add 4 mL of glacial acetic acid to 100 mL of filtered *Harris hematoxylin stock solution*.

Eosin Y stock solution (1%): Add 1.0 g of eosin Y to 100 mL of distilled water and mix well. [Note—This solution can be stored for up to 1 year at room temperature.]

Phloxine B stock solution: Add 1.0 g of phloxine B to 100 mL of distilled water and mix well. [Note—This solution can be stored for up to 1 year at room temperature.]

Eosin–phloxine B working solution: Add 100 mL of *Eosin Y stock solution (1%)* and 10 mL of *Phloxine B stock solution* to 780 mL of 95% alcohol, then add 4 mL of glacial acetic acid and mix well. [Note—This solution can be stored for up to 1 year at room temperature.]

Samples: 5-mm × 5-mm pieces of Scaffold Human Amniotic Membrane Allograft prepared as follows. Place each piece of trimmed tissue into a pre-labeled tissue cassette. Place the cassettes into a tissue processor cassette basket that is set into a container of 10% formalin.

Tissue processing: Tissue processing can be done using an automated tissue processor programmed using a stepwise schedule with all steps done under a vacuum, except the first 10% neutral formalin step. The stepwise procedure is provided in *Table 2*.

Table 2

Step Solution	Time	Retort Temperature
10% neutral formalin	10 min	ambient
70% alcohol	15 min	ambient
80% alcohol	15 min	ambient
95% alcohol	15 min	ambient
95% alcohol	20 min	ambient
Absolute alcohol	15 min	ambient
Absolute alcohol	15 min	ambient
Absolute alcohol	20 min	ambient
Xylene	15 min	ambient
Xylene	20 min	ambient
Paraffin	20 min	61°
Paraffin	20 min	61°
Paraffin	20 min	61°

Embedding: Processed cassettes are placed into the embedding cassette bath and are embedded in paraffin–polyisobutylene embedding medium[2] on an on-edge orientation.

Sectioning: Blocks are sectioned at 5 µm using a microtome. Sections are floated on a water bath containing distilled water at a temperature of approximately 47°. A single section is placed on a charged slide. Multiple slides may be generated for additional stains. Before staining, slides are dried for 15 ± 5 min at 60 ± 5° in the slide drying oven.

Staining: Fixed *Samples* on slides are stained using the following procedure.

1. Deparaffinize sections, 2 changes of xylene, 5 min each
2. Re-hydrate in 1 change of absolute alcohol for 3 min
3. Re-hydrate in 95% alcohol for 2 min
4. Wash in running water for 3 min
5. Stain in *Harris hematoxylin working solution* for 10 min
6. Wash in running tap water for 5 min
7. Differentiate in 1% acid alcohol and 30% water containing 1% hydrochloric acid (70% for 15 s)
8. Wash in running tap water for 5 min
9. Stain with blue in ammonia water [1% solution of ammonium hydroxide (28%–30%)] for 6 s
10. Wash in running tap water for 5 min
11. 95% alcohol for 6 s
12. Counterstain in alcoholic eosin (0.25% eosin in 80% alcohol and 20% water) for 5 min
13. Differentiate in 95% alcohol for 10 s
14. Dehydrate in absolute alcohol for 30 s
15. Dehydrate in 2 changes of absolute alcohol, 2 min each
16. Clear in 2 changes of xylene, 1 min each
17. Hold in xylene for 2 min

Coverslipping and mounting: Stained slides are removed from xylene and are coverslipped. The appropriate size coverglass is placed with a suitable mountant using a volume appropriate for the selected coverglass size. When complete, the slides are removed, placed into slide flats and allowed to dry.

Analysis: Scaffold Human Amniotic Membrane Allograft preparations are identified as thin strips, NMT 100 µm of eosinophilic membranous material. The structure of the tissue includes distinct amniotic basement membrane with the bulk of the preparation appearing as dense, eosinophillic acellular matrix. If any residual amnion cells are present, the cells align on the surface of the membrane. Identify the presence of cell nuclei and cytoplasm in the samples:

1. Cell nuclei: stain blue
2. Cytoplasm: stains pink
3. Erythrocytes: stain red

The presence of cells is noted for each tissue sample. Tissue samples are examined and the number of cells per 2.5-mm length of tissue is counted. [Note—*Samples* are sterilized with e-beam radiation with a certified dose sufficient to render any remaining eukaryotic cells non-viable.]

Acceptance criteria: NLT 99% cell free or NMT 64 cells/mm^2 or NMT 2 cells/*Sample*. No bacterial cells identified by abnormal blue inclusions can be detected in the observed field. No inflammatory inclusions (such as granuloma) can be present in the *Sample*. No malignant cells, hyperplastic cells, benign tumor cells, or other cell debris can be present in the *Sample*.

SPECIFIC TESTS

• **Hydroxyproline Assay for Collagen Content**

10X Assay buffer (10X acetate/citrate assay buffer): Weigh 50 g of citric acid, 120 g of sodium acetate trihydrate, and 34 g of sodium hydroxide. Add 890 mL of deionized or sterile water, 12 mL of glacial acetic acid, and 50 µL of toluene. Mix solution until it is free of visible particles, and then pass through a 0.22-µm filter. This buffer is stored at 2°–8° and is stable for at least 1 month.

1X Assay buffer (1X acetate/citrate assay buffer): Dilute 100 mL of *10X Assay buffer* with 900 mL of deionized or sterile water, and mix until solution is homogenous. Pass one time through a 0.22-µm filter. This buffer is stored at 2°–8° and is stable for at least 1 month.

Chloramine T reagent: Accurately weigh 4.23 g of chloramine T hydrate. Add 53.3 mL of *10X Assay buffer*, 20.7 mL of sterile water, and 26.0 mL of 1-propanol. Mix for approximately 1 h until solution is clear. This reagent is stored at room temperature and is stable for at least 12 h.

DMAB reagent: Weigh 12.0 g of 4-(dimethylamino) benzaldehyde (DMAB). Add 48 mL of 1-propanol and 20.7 mL of 70% perchloric acid, and then mix until the solution is free of visible particles. Solution is light sensitive and should be covered with foil until use. This reagent is stable at room temperature for at least 12 h after preparation.

System suitability solutions: Weigh 250 ± 5 mg of *trans*-4-hydroxy-L-proline. Calculate the volume of 6.0 N hydrochloride needed to create a 1000 µg/100 µL hydroxyproline solution and then, using the density of 6.0 N hydrochloride, which is 1.06 g/mL at 20°, determine the mass

of that volume. Weigh the previously calculated mass of 6.0 N hydrochloride into an appropriate container, and then mix in the pre-weighed hydroxyproline reference material creating the 1000 µg/100 µL hydroxyproline solution.

High system suitability solution: 22.5 µg/200 µL prepared as follows. Weigh 121.476 g of 6.0 N hydrochloride and mix with 5.40 mL of 1000 µg/100 µL hydroxyproline solution.

Middle system suitability solution: 14.5 µg/200 µL prepared as follows. Weigh 123.511 g of 6.0 N hydrochloride and mix with 3.48 mL of 1000 µg/100 µL hydroxyproline solution.

Low system suitability solution: 6.5 µg/200 µL prepared as follows. Weigh 125.546 g of 6.0 N hydrochloride and mix with 1.56 mL of 1000 µg/100 µL hydroxyproline solution.

The *System suitability solutions* are stored in 2-mL aliquots at 2°–8° and are stable for at least 1 month.

Hydroxyproline stock solution: Purified hydroxyproline (1 mg/mL) in 0.01 N hydrochloride prepared as follows. Weigh 150 ± 5.0 mg of *trans*-4-hydroxy-L-proline reference material into a glass vial. Add a calculated volume of 0.01 N hydrochloride to create a 50-mg/mL hydroxyproline solution and mix until homogenous. Add 2.0 mL of 50-mg/mL hydroxyproline solution to a 100-mL volumetric flask, and add 0.01 N hydrochloride to volume. Invert the flask 7–10 times to ensure solution is homogenous. This solution is stored in 5.0-mL aliquots at 2°–8° and is stable for at least 1 month.

Standard solutions: Remove a single vial of the *Hydroxyproline stock solution* (1 mg/mL in 0.01 N hydrochloride) from storage and dilute 8 times in 0.01 N hydrochloride to a final concentration of 25 µg/200 µL. This working standard is the highest reference point in the hydroxyproline calibration curve. Prepare additional standard solutions by diluting the working standard with 0.01 N hydrochloride to final concentrations of 20, 16, 12, 8, and 4 µg per 200 µL. Include a 0.01 N hydrochloride blank in the assay as a background control.

Samples: Weigh NLT 3 samples, 5 ± 0.5 mg each, of Scaffold Human Amniotic Membrane Allograft. Place the samples in individual glass vials, and add 2 mL of 6.0 N hydrochloride to each vial so that the solution covers the material. Hydrolyze the samples by placing the vials on a dry heat block for 18 ± 1 h at 110°. After hydrolysis is complete, remove the vial caps and dry the samples uncovered at 110° for an additional 3–4 h. Resuspend the concentrated hydrolysate in 4.0 mL of 0.01 N hydrochloride for assay use.

System suitability requirements: The standard curve (R^2) must be NLT 0.98. The percent bias of the standard curve must be NMT 20% for 75% of all standard points (14 of 18). The percent coefficient value of the standard curve must be NMT 20% for 75% of all standard points. The percent bias of the *High system suitability solution*, *Middle system suitability solution*, and *Low system suitability solution* is NMT 20%. The percent coefficient value of the *High system suitability solution*, *Middle system suitability solution*, and *Low system suitability solution* is NMT 20%.

Analysis: Triplicate samples (100 µL) are added to individual glass vials labeled with the corresponding *Sample* weight (mg). Add 100 µL of 0.01 N hydrochloride to each *Sample* vial. To generate the hydroxyproline standard curve, 200 µL of each *Standard solution* is pipetted, in triplicate, into individual glass vials and 1.8 mL of *1X Assay buffer* is added to each *Sample* and hydroxyproline standard, bringing the final volumes to 2.0 mL. The hydroxyproline is oxidized by incubating the samples at room temperature for 30 min in 1.0 mL of *Chloramine T reagent*. At the end of this reaction, add 1.0 mL of *DMAB reagent* to each vial and allow to incubate at 60° for an additional 30 min. During this time, the *DMAB reagent* reacts with oxidized hydroxyproline residues, generating a colorimetric product that is directly proportional to the amount of hydroxyproline present in the test samples. At the conclusion of this reaction, transfer 200 µL of each *Standard solution* and *Sample* to individual wells of a clear, flat-bottom 96-well plate. Acquire data by spectrophotometer at an optical density (OD) of 550 nm. Hydroxyproline levels in test samples are determined by interpolation against the hydroxyproline standard curve using suitable software or by plotting.

Calculate the total amount of collagen:

$$\text{Result} = \{[(H_p/V_a) \times V_S]/F_1\}/F_2$$

H_p = hydroxyproline value interpolated from the standard curve (µg)

V_a = volume used in the assay (µL)

V_S = volume of 0.01 N hydrochloride used to resuspend the concentrated hydrolysate, 4000 µL

F_1 = standard factor, mass ratio of hydroxyproline residue in collagen, 0.125

F_2 = unit conversion factor, 1000 µg/mg

Calculate the percentage of collagen (%):

$$\text{Result} = (T/W) \times 100$$

T = total amount of collagen, previously calculated

W = *Sample* weight

Acceptance criteria: Collagen content measured by hydroxyproline content is 74%–94%.

- **Elastin Content**

Diluent: 200 mL of 85% (v/v) 1-propanol in water prepared as follows. Mix 30 mL of sterile water with 170 mL of 1-propanol.

Dye dissociation reagent: 3 M guanidine hydrochloride in *Diluent* prepared as follows. Weigh 28.7 ± 0.5 g of guanidine hydrochloride and add into 100 mL of *Diluent*. Stir at room temperature until completely dissolved (approximately 30 min). Wrap with aluminum foil. This reagent is stable for at least 1 month at room temperature.

Precipitating reagent: 15% (w/v) trichloroacetic acid in hydrochloride prepared as follows. Weigh 15 ± 1 g of trichloroacetic acid, add 60 mL of sterile water, and stir to dissolve. Add 20 mL of 0.06 N hydrochloride to make a solution with a pH of −0.3 ± 0.1. Add additional sterile water to 100 mL. Wrap the bottle with aluminum foil; store at 2°–8°. This reagent is stable for at least 3 months at a storage temperature of 2°–8°.

0.4 M phosphate buffer: Mix 320 mL of 1 M phosphate buffer stock solution, pH 7.4, with 480 mL of sterile water. This solution is stable for at least 3 months at a storage temperature of 2°–8°.

Saturated ammonium sulfate buffer: Dispense 200 mL of *0.4 M phosphate buffer* into an appropriate container. Add 150 ± 1 g of ammonium sulfate. Stir at room temperature for 30 min. The top solution phase is ready to use after undissolved ammonium sulfate settles completely. This solution is stable for at least 3 months at 2°–8°.

0.4 M phosphate buffer with 24% saturated ammonium sulfate: Prepare 600 mL as follows. Mix 144 mL of *Saturated ammonium sulfate buffer* with 456 mL of *0.4 M phosphate buffer*. This solution is stable for at least 3 months at 2°–8°.

Dye reagent: 0.5 mg/mL of 5,10,15,20-tetraphenyl-21*H*, 23*H*-porphine-*p*, *p*',*p*'',*p*'''-tetrasulfonic acid tetrasulfonate (TPPS) in *0.4 M phosphate buffer with 24% saturated ammonium sulfate*. Weigh 0.250 ± 0.05 g of TPPS powder. Add the weighed powder into 500 mL of *0.4 M phosphate buffer with 24% saturated ammonium sulfate*. Stir at room temperature until completely dissolved (approximately 15 min). Pass TPPS dye solution through a 0.22-µm filter. Wrap the bottle with aluminum foil. This reagent is stable for at least 1 month at 2°–8°.

Assay diluent: 0.25 M oxalic acid prepared as follows. Mix 1 part 0.5 M oxalic acid with 1 part deionized water. Store at 15°–30°.

High system suitability solution: 25 µg/250 µL of tropoelastin in *Assay diluent*

Middle system suitability solution: 20 µg/250 µL of tropoelastin in *Assay diluent*

Low system suitability solution: 15 µg/250 µL of tropoelastin in *Assay diluent*

Tropoelastin standard stock solution: 0.200 mg/mL of human recombinant tropoelastin in 0.25 M oxalic acid prepared as follows. Weigh 11 ± 1 g of tropoelastin (combine vials as needed), and place in a labeled glass vial. Record the weight. Calculate the amount of *Assay diluent* needed to make 4 mg/mL with the amount of tropoelastin measured above (e.g., 2.5 mL of oxalic acid is needed for 10.0 mg of tropoelastin). Record the volume. Add the calculated amount of *Assay diluent* to the labeled glass vial using a calibrated pipettor. Mix 6 times on a vortex mixer for 20 s each time at 3000 rpm. Invert the vial 3 times between each vortex. Visually check if tropoelastin is completely dissolved. If it did not dissolve, repeat previous step. Further dilute with *Assay diluent* the reconstituted 4 mg/mL tropoelastin solution to 0.200 mg/mL using a volumetric flask. Store at −20 ± 2°.

Standard solutions: Remove a single 2.2-mL vial of the *Tropoelastin standard stock solution* (0.200 mg/mL in *Assay diluent*) from storage at −20 ± 2°, thaw at room temperature, and dilute in *Assay diluent* to the following concentrations: 30, 27, 22, 17, 12, 10, and 8 µg per 250 µL. A blank of *Assay diluent* is included in the assay as a non-specific background control.

Sample: Weigh NLT 3 samples, 5.0 ± 1.0 mg each, of Scaffold Human Amniotic Membrane Allograft and place into individual glass vials. Add 2.5 mL of *Assay diluent* to each vial so that the solution covers the sample. Hydrolyze the samples by placing the vials on a dry heat block for 30 ± 5 min at 100°. Centrifuge the samples, and remove and set aside the supernatant. Repeat the hydrolysis step a second time, and pool the resulting supernatants. Store the pooled hydrolysate at 2°–8° until processed in the assay.

System suitability requirements: The standard curve (R^2) must be NLT 0.98. The percent bias of the standard curve must be NMT 20% for 75% of all standard points. The percent bias of the *High system suitability solution*, *Middle system suitability solution*, and *Low system suitability solution* is NMT 30% of each level's target concentration. The percent coefficient value of the *High system suitability solution*, *Middle system suitability solution*, and *Low system suitability solution* is NMT 20%.

Analysis: To each of 3 individual, labeled, microcentrifuge tubes, add 65 µL of the pooled hydrolysate sample. Add 185 µL of *Assay diluent* to each sample tube. To generate the tropoelastin standard curve, pipette 250 µL of each *Standard solution*, in triplicate, into individual microcentrifuge tubes. Thaw the *Standard solutions*, and transfer 250 µL of each into individual microcentrifuge tubes, in triplicate. Add 250 µL of pre-chilled *Precipitating reagent* (1:1) to all test samples and standards, and incubate at room temperature for 10 min. Centrifuge all samples and standards, and discard the supernatants. Add 1 mL of elastin dye reagent (5,10,15,20-tetraphenyl-21,23-porphine tetra-sulfonate or TPPS), which complexes with solubilized elastin, to the test samples and standards. Mix all the vials vigorously on a vortex mixer, and incubate for 120 min on an orbital shaker at room temperature. Subsequently, centrifuge the samples and standards, and discard the supernatants. Add 250 µL of elastin *Dye dissociation reagent* to the pellet (to dissociate the dye from the solubilized elastin), and incubate for 10 min at room temperature. Centrifuge the samples and standards briefly to pull down all condensate off of the microcentrifuge tube walls, and then transfer the full 250 µL from each tube to a 96-well microplate. Data is acquired by spectrophotometer at an optical density (OD) of 513 nm. Determine the elastin levels in the test samples by interpolating against the tropoelastin standard

curve, using an absorbance plate reader and associated software capable of reading absorbance. Additional data analysis is performed using statistical analysis software.

Calculate the total amount of elastin in mg:

$$\text{Result} = (M_E/V_S) \times V_T \times F$$

M_E = measured mass of elastin (µg)

V_S = volume of pooled hydrolysate *Sample*, 65 µL

V_T = total volume of elastin extract for each *Sample*, 5000 µL

F = conversion factor, 0.001 (µg to mg)

Calculate the percentage of elastin (%):

$$\text{Result} = (E/S_W) \times 100$$

E = total amount of elastin (mg)

S_W = sample weight (mg)

Acceptance criteria: 9.0%–17.0% elastin content

- **Bacterial Endotoxins Test ⟨85⟩**

Sample: Prepare 2 cm × 3 cm of Scaffold Human Amniotic Membrane Allograft as follows. Extract the sample by immersing in endotoxin-free water and placing it on an orbital shaker in an incubator for NLT 60 min at 37°–40°.

Acceptance criteria: NMT 3.3 USP Endotoxin Units/*Sample*

- **Sterility Tests ⟨71⟩**

Sample: 1 cm × 2 cm of Scaffold Human Amniotic Membrane Allograft

Analysis: Test NLT 5 *Samples* in both *Soybean–Casein Digest Medium* and *Fluid Thioglycollate Medium* at an incubation temperature of 30°–35° and 20°–25°, respectively.

Acceptance criteria: Meets the requirements

- **Water Determination ⟨921⟩, *Method I, Method Ia***

Sample: Prepare 6 cm × 6 cm of Scaffold Human Amniotic Membrane Allograft as follows. Place the sample in a dry vial. Seal the vial with an aluminum seal and a cap and place it in a drying oven where the water content is thermally released and transferred to a titration vessel by means of a dried, inert purge gas.

Analysis: Proceed as directed in the chapter.

Acceptance criteria: NMT 20%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Scaffold Human Amniotic Membrane Allografts range in size from 1 cm × 2 cm to 6 cm × 6 cm per batch. Scaffold Human Amniotic Membrane Allografts are packaged in polyester-based heat-sealed laminate film peel pouches, supplied as a single-use, sterile, membrane sheet, and stored at room temperature.

• **Labeling:** The package label indicates the size of the Scaffold Human Amniotic Membrane Allograft, the lot number, the expiration date, the trade name, the required storage conditions, the dosage information, and manufacturer and/or distributor contact information. “Instructions for Use”, which includes a summary of records used to make donor eligibility determination and necessary information for properly using the product for its intended use, is provided with each allograft.▲2S *(USP41)*

[1] A suitable reagent can be obtained from VWR, catalog #95057-858, or use a suitable equivalent.

[2] A suitable embedding and infiltration medium can be obtained from McCormick, catalog #39501006, or use a suitable equivalent.

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
SCAFFOLD HUMAN AMNIOTIC MEMBRANE ALLOGRAFT	Rebecca C. Potts Associate Scientific Liaison	BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines

Topic/Question	Contact	Expert Committee
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	BIO32020 Biologics Monographs 3 - Complex Biologics and Vaccines

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